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AN IMPROVED METHOD FOR RECOVERING TRYPANOSOMES FROM THE BLOOD OF RATS FOR ANTIGEN PURPOSES IN CONNECTION WITH COMPLEMENT FIXATION

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UNDESIRABLE FEATURES OF METHODS IN USE

Up to the present time the methods employed in recovering trypanosomes from the blood of artifically infected rats, though quite satisfactory, have presented some features which were somewhat detrimental to the antigen and which, if overcome, would greatly improve the same as to purity and, obviously, specificity. The question of suitable and sufficient antigens being one of vital importance, more especially in this laboratory, where many thousands of fixation tests for dourine are performed annually, it was desirable to seek an improvement.

Watson [1] describes a method whereby the trypanosomes might be recovered in large quantities from the blood of infected rats killed at the height of the disease. It was essentially a method of repeated centrifuging, as the erythrocytes, being of greater specific gravity than the trypanosomes, necessarily settled to the bottom. However, the method had its faults, in that many of the organisms would be drawn down with the red cells and it was necessary to sacrifice many of the trypanosomes to prevent the presence of too great a quantity of red cells in the antigen. Therefore it was necessary to eliminate the following undesirable features: First, however careful and painstaking one might be, it was quite impossible to collect all the trypanosomes present in the blood; second, it was impossible to procure a pure antigen totally devoid of rat erythrocytes which, when present in large numbers, would tend to decrease the antigenic value as well as the keeping qualities; third, it was a laborious process consuming half a day, and, as experience has shown that subjection to room temperature greatly impairs the antigenic value, the necessity for its rapid preparation and early storage on ice is quite apparent. Even then it was quite impossible to procure a pure product. There-

[1] WATSON, E. A. DOURINE AND THE COMPLEMENT FIXATION TEST. *In* Parasitology, v. 8, no. 2, p. 156–183. 1915.

fore, with a view to overcoming the several obstacles attending the preparation of the trypanosomal antigen, the following technic has been devised and has given good results.

TECHNIC OF NEW METHOD

Blood of infected rats is collected in a 1 per cent sodium-citrate solution in physiological salt solution in order to prevent coagulation. When all the blood has been collected, the solution is filtered through cheesecloth to remove clots, fibrin, etc., poured into tubes, and centrifugalized for about 20 minutes at 2,100 revolutions per minute. This precipitates all the corpuscles and most of the trypanosomes, leaving an upper stratum of blood serum and citrate solution containing some of the organisms. This fluid is drawn off and again centrifugalized in order to recover any of the protozoa which may be present. To the other tubes containing the mass of corpuscles intermixed with and superimposed by trypanosomes is added sufficient distilled water to produce complete hemolysis of the rat erythrocytes, a matter of about 20 minutes, which procedure is facilitated by agitation of the mixture in a flask. This also is centrifugalized but, in this instance for about half an hour, upon the completion of which there is found at the bottom of the tubes a mass of trypanosomes with an admixture of stroma of the hemolyzed red cells, which latter, in quantity, has been found to be negligible. After discarding the supernatant fluid (hemoglobin-stained water) physiological salt solution is added and the material vigorously shaken until the mass of trypanosomes is disintegrated and evenly distributed throughout the solution. Centrifuging is again resorted to with similar results, the washed mass of trypanosomes being packed at the bottom of the tubes. The salt solution is poured off and an amount of preserving fluid (physiological salt solution and glycerin aa) equal to about twice the amount of trypanosomes added; the mixture is then agitated until a uniform suspension is acquired, when it is stored at a low temperature until used.

In order to determine whether the use of distilled water in laking the corpuscles would have any detrimental effect on the trypanosomes as regards their antigenic value, immediately following their preparation, and also after an interval of two weeks, the following procedure was employed:

Twenty white rats were inoculated subcutaneously with a suspension of *Trypanosoma equiperdum* in salt solution. At the end of the third day the blood of all the animals showed a heavy infestation with the protozoa.

The animals were then bled to death into 300 cc. of a 1 per cent sodium-citrate solution in physiological salt solution, which was then divided equally and placed into two flasks.

The trypanosomes in flask 1 were recovered by repeated centrifugalization, the machine being run a sufficient length of time to drive all the corpuscles to the bottom of the tube, but leaving a large number of trypanosomes in the supernatant fluid. This was pipetted off and the blood mixed with salt solution and again centrifugalized. By repeating this operation a number of times, as many trypanosomes as possible were obtained in suspension. This fluid was then centrifugalized, driving all the trypanosomes to the bottom of the tube. The supernatant fluid was discarded and the trypanosomes were suspended in the following solution: Glycerin 5 cc., physiological salt solution 5 cc., and labeled "Antigen I."

The trypanosomes in the second flask were recovered by breaking up the red blood cells with distilled water in the manner hereinbefore described. These trypanosomes were also suspended in 5 cc. of glycerin and 5 cc. of physiological salt solution and labeled "Antigen II."

On inspection of the two antigens it was readily seen that Antigen II contained many more trypanosomes than Antigen I and apparently no blood corpuscles, while Antigen I showed the presence of considerable blood which it had been impossible to get rid of without sacrificing many of the trypanosomes.

Both antigens were titrated for antigenic strength and anticomplementary action immediately after preparation and after being stored in the ice box for two weeks. Tests for hemolytic action were also made.

The hemolytic system employed consisted of a 3 per cent suspension of sheep red cells, 2½ units of hemolytic amboceptor, and 1½ units of complement, the latter being titrated against the amboceptor and sheep cells.

Both antigens were diluted 1 to 10 with physiological salt solution.

The test for antigenic power was made against 0.15 cc. of known positive dourine serum, which was the pooled serum from 20 horses affected with dourine.

In the test immediately after preparation the antigenic unit of Antigen I was 0.25 cc. and the anticomplementary unit was 2 cc., and was not hemolytic in five times this amount. The antigenic unit of Antigen II was 0.05 cc., the anticomplementary unit 3 cc., and was not hemolytic in five times this amount.

Two weeks after preparation the antigenic unit of Antigen I was 0.35 cc., the anticomplementary unit 1.5 cc., and the antigen showed no hemolytic action. The antigenic unit of Antigen II was 0.1 cc., the anticomplementary unit 3 cc., and it showed no hemolytic action.

The results are compared in the following table:

Antigen.	Antigenic unit.	Anticomplementary unit.	Hemolytic action.
Immediately after preparation:	*Cc.*	*Cc.*	
Antigen I	0.25	2.0	None.
Antigen II	.05	3.0	None.
Two weeks after preparation:			
Antigen I	.35	1.5	None.
Antigen II	.10	3.0	None.

The use of distilled water in laking the red blood cells had no detrimental effect on the trypanosomes with regard to their antigenic value.

CONCLUSIONS

In concluding, the following advantages of the new method are pointed out:

(1) The antigen is freed of all erythrocytes.
(2) All the trypanosomes present in the blood are recovered.
(3) The keeping quality is improved.
(4) The time consumed is about one and one-half hours, with practically no effort, as compared with four or five hours.
(5) The antigenic power is increased and the anticomplementary action diminished.

LIFE HISTORY OF PEMPHIGUS POPULI-TRANSVERSUS

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With technical descriptions by C. P. Gillette, *Collaborator, State Entomologist of Colorado*

INTRODUCTION

The first collection in Louisiana of aphids belonging to the genus Pemphigus from the roots of plants of the family Cruciferae seems to have been made on November 19, 1914, by Mr. E. S. Tucker, formerly Associate Entomologist of the Louisiana Experiment Stations. These specimens were taken from cabbage roots (*Brassica oleracea capitata*) at Loranger, in Tangipahoa Parish. Shortly afterwards root aphids of the same genus were noted at Baton Rouge by the writer, and material for identification was submitted to specialists acquainted with the genus. As the stages sent were not recognized as belonging to any described species, and as there were apparently no published records of any species of Pemphigus occurring on crucifers in the United States, studies concerning the life history, habits, and economic importance of the species were begun.

After the writer had begun the investigation Maxson (*23, p. 501*)[2] published an article on *Pemphigus betae* Doane in which he stated that—

> Lice of this genus have been repeatedly taken on turnips in the south.

In a letter to the writer Mr. Maxson has given the additional information that—

> the specific instances referred to were brought to my attention by Mr. F. B. Paddock, State Entomologist of Texas, he having sent material to me for identification.

[1] The writer wishes not only to thank Dr. Gillette for his kindness in drawing up the descriptions and supervising the preparation of illustrations, but also for suggestions regarding the biological studies, and especially for the interest which he has taken in the work. Mr. J. J. Davis, of the Bureau of Entomology, and Mr. J. R. Parker, of the Montana Agricultural Experiment Station, also have shown much interest in these studies, and the writer desires to thank them for the aid they have given. Messrs. C. E. Smith and J. L. E. Lauderdale, while members of the Bureau of Entomology, rendered valuable assistance in the studies of the life history and habits of the species.

[2] Reference is made by number (italic) to "Literature cited," p. 592–593.

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Dr. C. P. Gillette also informed the writer that Mr. Paddock sent him material from Texas collected from turnip roots on February 13, 1914, and that the species is the same as that which attacks crucifers in Louisiana. Mr. L. C. Bragg has collected the species from watercress (*Roripa* sp.) near Fort Collins, Colo. Mr. J. J. Davis, of the Bureau of Entomology, has written that several years ago he received a species of Pemphigus collected on curly turnip (*Brassica rapa*) at Greenwood, Miss., which seems to agree with the one under discussion.

It is the purpose of this paper to present the results of investigations carried on at Baton Rouge regarding the life history and habits of the species, together with records from other points in Louisiana and from other States. These results indicate that the species of Pemphigus that feeds on the roots of crucifers is identical with the well-known *Pemphigus populi-transversus* Riley, which causes galls on the leaf petioles of some of the poplars or cottonwoods (*Populus* spp.). Fortunately, it has been possible to have these notes accompany descriptions of the various stages made by Dr. Gillette.

HISTORICAL REVIEW

While *Pemphigus populi-transversus* has been often referred to by entomological writers since Riley described the species in 1879 (*1, p. 15–16; pl. 2, fig. 5, a–c*), these references have been brief and for the most part have been limited to records of the occurrence of the species in some State or particular locality and a few words regarding bibliography and the previously recorded hosts and distribution. Such references are mentioned under "Distribution and hosts" on page 580.

In works in which insects are treated especially from an economic standpoint there are several references to the species and its gall. In 1890 Packard (*3, p. 434*) mentioned it in his work, "Insects injurious to forest and shade trees." Bruner (*6, p. 218, fig. 57*), included it in an article entitled "Insect enemies of ornamental and shade trees growing in cities and parks," which was published in 1893 in the Annual Report of the Nebraska Horticultural Society, and again mentioned it (*11*) in an article on aphids which appeared in The Nebraska Farmer in 1901. Lintner (*9, p. 361–362*) included it in his Thirteenth Report of the State Entomologist of New York, published in 1898, and stated that it had been abundant on *Populus monilifera* in Washington Park, Albany, N. Y., during 1896 and 1897. Felt (*14, p. 247, 620, 635–636, pl. 11, fig. 15, 16*) in his work, "Insects affecting park and woodland trees," published by the New York State Museum in 1906, also referred to its abundance in the vicinity of Albany and gave colored figures of the gall. Baldwin (*21, p. 208*) referred to it in the Fifth Annual Report of the State Entomologist of Indiana in 1912 and gave two original illustrations of the gall.

In lists and synopses of North American aphids the species has been mentioned by several authors. It is included in the "Host-plant list of North American Aphididae" by Williams (*4, p. 6, 9*), published in 1891, and in a bulletin entitled "The Aphididae of North America" by Hunter (*10, p. 78*), issued in 1901. In a synopsis of the genus Pemphigus Jackson (*14, p. 182, 183, 206–208*) in 1908 referred to the species somewhat in detail and stated that the life history was "very imperfectly known." Further references to its life history have been made by Davidson (*16, p. 372*) and Gillette and Bragg (*22, p. 98*). Davidson stated that the stem mothers had been observed "founding their colonies" in the vicinity of Stanford University, California, in March. Gillette and Bragg (*22, p. 98*) in "Notes on some Colorado aphids having alternate food plants," published in 1916, gave "winter host, Populus species; alternate host unknown."

EXPERIMENTS IN TRANSFERRING THE SPECIES FROM CRUCIFERS TO POPLAR AND FROM POPLAR TO CRUCIFERS

Soon after investigational work on this root aphid was begun, winged viviparous females (winged migrants or sexupara), collected from the soil about cruciferous roots, were sent to Dr. Gillette and to Mr. J. R. Parker. Both stated that they did not recognize the individuals as belonging to any described species of the genus Pemphigus, but suggested that it was possible that they might be identical with *Pemphigus populi-transversus*. In view of this, experiments were begun in an attempt to ascertain whether there was a migration of the species from crucifers to poplar at one season of the year, and a return migration at another season.

In 1916 cuttings were taken from trees of *Populus deltoides*, before the buds began to swell, and stuck in moist sand in flowerpots, which were kept in a greenhouse. When leaves began to appear on these cuttings, young individuals of the stem mother (fundatrix) were placed on them. These stem mothers had recently issued from eggs obtained in the laboratory from the true sexes, which had in turn been produced by winged females (sexupara) taken from about the roots of crucifers in the field. Swellings soon began to appear on the petioles where the stem mothers had located, and these swellings, increasing in size, gradually took on an appearance typical of the gall of *Pemphigus populi-transversus* (Pl. 82). Unfortunately the stem mothers died after the galls had reached a diameter of about ⅜ inch. Galls were again formed about stem mothers on leaf petioles of poplar in the greenhouse in the spring of 1917, and these, developing to a greater size, appeared identical to those of *P. populi-transversus*.

During the fall of 1916 winged migrants (fundatrigenia) from the galls of *Pemphigus populi-transversus* were placed under cheesecloth in a cage where turnips were growing. Later, examination of the soil showed the roots of the turnips to be heavily infested with aphids of the genus

Pemphigus, the infestation being similar to that which occurs on cruciferous roots under field conditions. It was evident, when the growth of the plants in this box was compared with that made by plants of the same age in an uninfested box, that they had been affected by the presence of the aphids at their roots (Pl. 84, F). In fact, the turnips died before winged migrants appeared, though probably not altogether because of the insect infestation.

Winged migrants from galls were also placed on turnips growing in pots in the greenhouse. The soil in these pots had been heated previously to a temperature sufficient to kill insect life, and the pots were covered with cloth-covered wire frames. Later, examination showed wingless individuals of *Pemphigus* sp. to be present on the roots, but again the plants died before the winged forms appeared. Before the plants died, however, a number of the wingless aphids were transferred to pots covered with cloth containing cabbage plants, the roots of which had been dipped in a mixture of water and nicotine sulphate before they were planted. On March 2 of the following year winged viviparous females, such as are found in the colonies at the roots of crucifers, were observed. No aphids appeared on the roots of control plants.

These experiments, together with others that have been carried on, indicate that the forms found on poplar and on crucifers belong to the same species. Additional proof is furnished by the fact that from poplar trees in the spring were taken winged viviparous females which agree with winged migrants found at cruciferous roots at that time of the year, and that during late summer and during the fall there were collected from the leaves of crucifers winged viviparous females which are identical with those found in galls of *P. populi-transversus*.

DISTRIBUTION AND HOSTS

The species has been recorded as occurring on poplar in California (*16, p. 372; 19, p. 398; 20, p. 699*), Texas (*1, p. 15–16*), Colorado (*1; 7, p. 116; 22, p. 98*), Kansas (*12, p. 22, 23*), Nebraska (*18, p. 12*), Missouri (*1, p. 15–16*), Iowa (*5, p. 130*), Minnesota (*2, p. 20, 21*), Illinois (*17, p. 411*), Indiana (*21, p. 208*), New York (*9, p. 361–362; 13, p. 247, 620, 635–636; 15, p. 355*), and Massachusetts (*15, p. 355*). Mr. Parker has collected it at Lovell, Wyoming, Dr. Gillette has specimens from Arizona, and Mr. Davis writes that he has records of its occurrence in Wisconsin, Michigan, and Ohio. Mr. H. F. Wilson has taken it in Wisconsin, and the writer has seen galls, apparently made by this species, at Agricultural College, Miss., and Jacksonvllle, Fla.

Four species of the genus Populus, *balsamifera*, *monilifera*, *trichocarpa*, and *fremontii*, have been mentioned as hosts. Britton and Brown (*8, p. 491, fig. 1165; p. 493, fig. 1172*) give the following distribution for *P. balsamifera:*

Newfoundland to Hudson Bay and Alaska, south to Maine, New York, Michigan, Idaho, and British Columbia.

They give *P. monilifera* as a synonym under *P. deltoides* (the name used in this article), a species which, they state, occurs from Quebec to the Northwest Territory, south to New Jersey, Florida, Colorado, and New Mexico. *P. trichocarpa* and *P. fremontii* apparently occur in the Western States.

FORMATION OF GALLS

In transferring the species from crucifers to sprouting cuttings of *Populus deltoides* it was found that the petioles of young leaves, just out of the bud, are apparently the only ones upon which galls begin to develop. A transverse groove first appears on the petiole where the young stem mother has located, the developing petiole gradually bending at this point. The tissue surrounding the groove, which is on the inside surface of the bent petiole, gradually enlarges until a hollow globular gall, with a transverse slit on the surface opposite the petiole, is formed around the stem mother. Galls found in the field on May 17 were roughly spherical in shape and varied in diameter from ¼ to ½ inch. Just before the leaves fall from the trees some of the galls reach a greatest diameter of nearly an inch. The galls vary considerably in shape, length of the transverse slit, and development of the lips (see Pl. 83; 84, A–E). While the general color of the gall is the same as that of the petiole, a portion of the surface often has a reddish tinge.

Practically all of the galls occur somewhere on the leaf petioles, though what seem to be the galls of this species have been found on the new stem growth to which the petioles are attached. The galls usually occur singly, but as many as three have been seen in juxtaposition on one petiole.

In midsummer the gall contains, and indeed is often filled with, the comparatively large stem mother, her progeny in various stages of development, all covered with waxy secretion, together with molted skins and usually with liquid globules.

DATES WHEN GALLS ARE FOUND AT BATON ROUGE

In 1917, at Baton Rouge, winged viviparous females and the true sexes produced by them were taken in a small cavity on the trunk of a poplar tree as early as March 8. These winged females are identical with the winged migrants (sexupara) found about the roots of crucifers. On March 28, young stem mothers were found on leaf petioles where, to judge from the size of the galls, they had apparently been present for a few days only.

During 1916, galls were noted on May 17 which had reached a diameter of ¼ to ½ inch. On November 7, when a large percentage of the leaves of the poplar had fallen, few galls remained on the trees.

PERCENTAGE OF LEAF PETIOLES SHOWING GALLS

Leaves were gathered from a tree of *Populus deltoides* on July 26, 1916, to ascertain the percentage of leaf petioles having galls upon them, and to learn whether galls occurred in greatest numbers in any one portion of the tree as regards the height above the ground. Galls are found upon comparatively small trees and upon the largest specimens.[1] The tree selected was about 30 feet in height and the infestation appeared to be an average one for the particular locality. Branches were broken from it at random at various heights. The leaves were then stripped from the branches and counts made of those leaves showing galls and those not infested. It was found that there was a considerable variation in the percentage of leaves showing galls on different branches, but the distance above the ground apparently had no bearing upon the percentage of leaves infested. The results are given in Table I.

TABLE I.—*Percentage of leaf petioles of Populus deltoides infested with Pemphigus populi-transversus, Baton Rouge, La., July 26, 1916*

Position of branch on tree.	Number of branches examined.	Number of leaves examined.	Number of leaf petioles showing galls.	Percentage of leaf petioles showing galls.
In approximate lower third	5	222	54	24.3
In approximate middle third	5	441	117	26.5
In approximate upper third	5	512	137	26.7
Total	15	1,175	308	25.2

DATES WHEN WINGED MIGRANTS (FUNDATRIGENIA) ARE FOUND IN GALLS

At Baton Rouge winged females begin to appear in the galls on *Populus deltoides* at a somewhat earlier date than that recorded for other localities. Riley (*1, p. 15–16*), in connection with his original description of the species, in which he gives Missouri, southern Texas, and Colorado as localities where this species of Pemphigus occurs, states that the winged females are "produced in autumn, sometimes not until the leaves have fallen." Williams (*18, p. 12*) mentions finding winged females, "evidently but lately transformed," in galls at Ashland, Nebr., on September 25. At Baton Rouge, in 1916, winged females were found in the galls as early as June 1, though a very small percentage of the galls contained such individuals on this date. Not until September were they present in more than 10 per cent of rather large collections examined.

[1] Three other species of aphids belonging to the subfamily Pemphiginae have been collected from *Populus deltoides* at Baton Rouge. Of these, *Pemphigus populicaulis* Fitch is the most common, although it is much less abundant than is *P. populi-transversus*. The two other species, found only occasionally in galls which they form on the leaves, have not been identified.

Of 200 galls collected on September 15, 17 per cent contained winged females, while they were present in all of 150 galls taken on September 28.

The number of winged females in a single gall also showed a gradual increase as the year advanced. In June usually only one was found in a gall, while on November 7 as many as 76 were present.

DATES WHEN WINGED MIGRANTS (FUNDATRIGENIA) LEAVE GALLS

Although winged females appeared in the galls at Baton Rouge during 1916 as early as June 1, there is no evidence to show that migration to crucifers took place until late summer. The earliest collection of winged female migrants on crucifer leaves was on August 31, and not until early October were crucifer roots found infested to any considerable extent. On October 2, 1917, during a period of clear, cool, autumn weather, the migrants from the galls were common on turnip leaves at least 500 feet from poplar trees. As many as five were found on the underside of a large leaf. The greatest migration probably occurs during October. While the writer has no definite data regarding the maximum distance they may traverse while in flight, it is probable that they, as well as the sexupara, may be carried long distances by winds.

NUMBER OF WINGLESS VIVIPAROUS FEMALES (VIRGOGENIA) TO WHICH WINGED MIGRANTS (FUNDATRIGENIA) GIVE BIRTH

On October 25, 1916, 25 winged females taken from galls were placed, without food, in vials and kept under observation indoors. All of these began almost immediately to give birth to young, and by October 30 all had died. The average number obtained from each individual was 26, the number ranging from 14 to 37. In one instance a female brought forth 30 young in about 24 hours.

The young viviparous females locate on the roots, feed, and when mature bring forth other wingless viviparous females. In this way the subterranean colonies become established.

INJURY TO AND APPEARANCE OF PLANTS INFESTED WITH SUBTERRANEAN FORMS

While severe Pemphigus infestation on the roots of crucifers may be indicated by the wilted condition of the leaves, a slight or moderate infestation does not usually affect, to a noticeable degree, the portions of the plant above the ground. For this reason such infestation usually goes unnoticed. In other words, this insect, while it may cause as much damage as many of those species which feed upon the leaves or other parts of the plant above the surface of the soil, does not attract as much attention as such species because it works out of the sight of the ordinary observer. Inasmuch as these aphids feed upon the roots, it is to be presumed, however, that any infestation is detrimental to the plant.

Upon examination of the soil about plants attacked by the subterranean forms colonies may be found upon any portion of the root system, but the small rootlets appear to be preferred. Where dead leaves, or other trash, occur on the surface of the soil, there is often a growth of rootlets immediately beneath, and colonies are often found in such locations.

Mr. J. L. E. Lauderdale made an interesting observation at Baton Rouge on March 19, 1917, while examining roots of *Coronopus didymus* in a field of stock beets (*Beta vulgaris*). The beets were growing on ridges where the soil was less moist and less compact than that midway between the rows. Of 25 plants of *C. didymus* growing on the ridges, 24 were infested with *Pemphigus populi-transversus*, whereas only 6 of 25 plants growing in the low ground between the rows had aphids present on their roots. This would indicate that either the compactness of the soil between the rows, or its higher moisture content, or both, was disadvantageous to the development of the aphids.

The white, flocculent material which the aphids secrete is of material aid in locating them. This secretion often occurs in considerable abundance about the colonies (Pl. 85). The light-yellow color, characteristic of the bodies of the wingless females, except in their early stages, usually makes it easy to locate them when the soil about the roots on which they are feeding is carefully examined.

INJURY CAUSED BY THE SUBTERRANEAN FORMS

Where the aphids occur in small or moderate numbers at the roots of plants, it is difficult to estimate the amount of damage done by them. The following extracts from correspondence received by the Louisiana Experiment Stations give information as to injury to crucifers by *Pemphigus* spp. in Louisiana. As only wingless forms were forwarded by the correspondents, it can not be stated positively that the root aphid causing the injury was *P. populi-transversus*, although that is probable.

On November 13, 1915, a correspondent living at Rhoda in St. Mary Parish wrote:

> I am sending you under separate cover a cabbage plant that is badly infected with a small yellow louse, and would ask if you can recommend anything that can be done. The bug or louse is found at the root of the plant, and seems to suck the sap or eat off the roots, as the plant is badly wilted during the warm part of the day, but revives a little at night, until it finally is killed.

On November 22 of the same year a letter was received from New Iberia, Iberia Parish, in which the writer stated:

> Enclosed please find stalk of cabbage with insect at the root that is destroying all my plants.

A report has also been received from Dr. C. E. Mauldin, in charge of the Iberia Live-Stock Experiment Farm of the Bureau of Animal Industry, at Jeanerette, La., that—

It has been necessary for us to abandon the planting of rape and kale at this station on account of the root-louse.

At Baton Rouge the subterranean forms apparently cause more severe injury to turnip than to any other cultivated cruciferous crop that has been under observation. Not only have they been found in greatest numbers on turnips but plants have been frequently noted which, when pulled, came up easily. The roots of these plants were mostly dead, apparently because of the attack of the aphids.

FOOD PLANTS OF THE SUBTERRANEAN FORMS

Wingless specimens of the genus Pemphigus have been taken in Louisiana from the roots of the following Cruciferae: Cabbage, turnip, mustard (*Brassica nigra*), cauliflower, and broccoli (*Brassica oleracea botrytis*), Brussels sprouts (*Brassica oleracea gemmifera*), rape (*Brassica napus*), *Coronopus didymus*, *Lepidium virginicum*, and *Roripa* sp. The last three host plants are weeds, the first being common in uncultivated fields at Baton Rouge during the winter months, when the plants are sometimes gathered and used as "greens."

Winged migrants (fundatrigenia) of the species of Pemphigus under consideration have been found at the roots of cabbage, turnip, Brussels sprouts, rape, *Coronopus didymus*, and *Roripa* sp. It is quite possible that further observations will disclose the fact that the species occurs also at the roots of plants not belonging to the family Cruciferae. Mr. Lauderdale has found individuals on the roots of stock beet. Adjoining infested roots of *Coronopus didymus* apparently explained their presence on the beet roots, as examination of the roots of many beets, near which no crucifers were growing, failed to disclose additional instances of infestation.

SPREAD OF SUBTERRANEAN FORMS

Observations made in the field and under laboratory conditions indicate that at least the smaller wingless viviparous females (virgogenia) that are present in the soil during the winter months are capable of considerable locomotion, and that when conditions become unsatisfactory, these individuals seek more suitable locations. During December they have been found in great abundance crawling over the surface of the soil and upon the plants in a field of Brussels sprouts. While carrying on some experiments in the greenhouse about a year later, individuals were found to have left flowerpots in which they were feeding on turnip roots, apparently because the turnips had begun to die as the result of being severely infested with the aphid *Myzus persicae* Sulzer. They were

especially numerous on the highest points of the cloth covering these pots, about a foot above the surface of the soil, and could be easily dislodged by slight puffs of air. Some were found under conditions which indicated that they were about 3 feet away from the nearest point where they could have originated.

As the season advances, the wingless viviparous females give birth to individuals which develop into winged viviparous females (sexupara), which later leave the soil and fly away.

DATES WHEN WINGED MIGRANTS (SEXUPARA) ARE FOUND IN THE SOIL

A few winged females have been found in the soil as early as December 12, and, as is the case with the winged females occurring in the galls, their number gradually increases as the season advances. During 1917, roots of *Coronopus didymus* were examined in the field from time to time with the idea of ascertaining how late in the spring the species occurs in the soil. The last winged individuals were taken on April 9. On April 16 no subterranean forms could be found, though winged migrants were alive on poplar as late as April 30.

From the field observations it appears that these winged migrants fly from the crucifers to the poplar trees during the spring, where, usually in some suitable crevice, they give birth to the true sexes. Winged females, agreeing with the winged females found about cruciferous roots, were found in such locations on poplar trees during March and April, 1917. Some observed on March 20 were dead, with true sexes and eggs located near by.

NUMBER OF YOUNG TO WHICH WINGED MIGRANTS (SEXUPARA) FROM CRUCIFERS GIVE BIRTH

In the laboratory, under conditions quite different from those under which the winged migrants would live in the field, the greatest number of sexed individuals to which a single aphid was observed to give birth was six. Usually this winged form brings forth all of her offspring within a short time and then dies. Those kept in the laboratory were examined daily, all of the young usually being produced from the time of one examination to the next. Individuals of both sexes have come from one winged migrant.

Examination of the abdomens of several winged migrants, collected from soil about the roots of crucifers, showed them to contain from four to seven sexed specimens, seven being the predominating number.

DEVELOPMENT OF THE TRUE SEXES (SEXUALES)

Eggs have been obtained in the laboratory from true sexes kept without food. The number of molts which the males and females undergo has not been ascertained, nor has it been learned when copulation takes

place. The female is larger than the male and deposits only a single egg. In a well-ventilated insectary at Baton Rouge, during 1916, eggs were first noted on March 6 in vials in which the true sexes had first appeared 12 days before.

OVIPOSITION

The egg is often found resting in a small amount of white, cottony material secreted by the female. The true sexes apparently do not, as a rule, move far from their places of birth, eggs being found in the field in crevices on the trunks and limbs of poplar trees where living winged migrants (sexupara) and the dead bodies of others were present. In the insectary at Baton Rouge stem mothers were first seen on March 22, 1916, in vials where eggs had first been noted on March 6, giving a period of 16 days for the incubation of the egg.

FORMATION OF GALL

Of necessity the young, active stem mother (fundatrix), after issuing from the egg, must make its way to the developing leaves, where it settles down on the petiole and becomes responsible for the formation of a gall. While there is no absolute proof that such is the case, it is believed from field observations that one stem mother is responsible for one gall only and that a gall is only formed when a young stem mother locates on a leaf petiole.

SEASONAL HISTORY OF PEMPHIGUS POPULI-TRANSVERSUS IN BRIEF

The following summary has been prepared from observations made in the field and laboratory at Baton Rouge (fig. 1). The dates when the various stages appear and migration takes place probably depend to some extent upon the weather. It would be interesting to know the seasonal history of the species in the northern portion of its range where climatic conditions, especially as regards temperature, are so different from those existing in Louisiana.

The galls begin to develop on the petioles of the young leaves of *Populus deltoides* in the spring. They increase in size during the summer and by the time the leaves fall in the autumn some have reached a diameter of nearly an inch.

Of 1,175 leaves gathered from a poplar tree on July 26, 1916, 26.2 per cent had galls on their petioles. They occur on both small and large trees.

Winged migrants (fundatrigenia) have been found in the galls as early as June 1. The percentage of galls containing winged migrants, as well as the number found in any one gall, increases as the season advances.

Winged migrants from the galls fly to various cruciferous plants. They have been found on the leaves of such plants as early as August 30 and as late as October 31.

The winged migrants give birth to viviparous females (virgogenia) which start colonies on the roots of crucifiers, upon which they feed.

The infestation at the roots of crucifiers, which is usually made apparent by the white, cottony material which the aphids excrete, gradually becomes more severe, owing to the increase in the number of the subterranean forms. It appears that, under certain conditions, the smaller, wingless viviparous females occurring in the soil are able to migrate to a considerable distance from their place of birth and there begin new colonies.

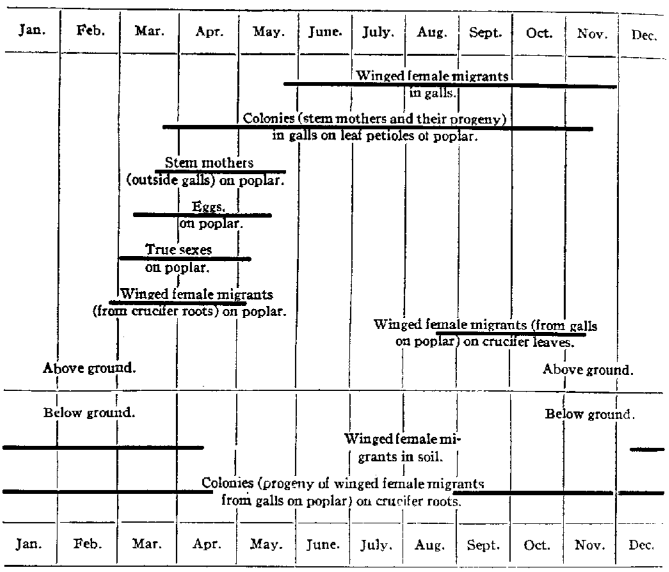

FIG. 1.—Diagram illustrating the seasonal history of *Pemphigus populi-transversus* at Baton Rouge, La.

Winged migrants (sexupara) appear in the subterranean colonies during the winter. They have been found at the roots of cabbage, turnip, Brussels sprouts, rape, *Coronopus didymus*, and *Roripa* sp. Colonies of a species of Pemphigus have also been found at the roots of mustard, cauliflower, broccoli, and *Lepidium virginicum*. No winged migrants were present in these colonies, but it appears probable that the aphids were of the same species.

In the spring the winged migrants fly from the crucifers to poplar trees where they give birth to the true sexes (sexuales), usually in crevices on the trunks and branches.

The sexed individuals take no food and, after pairing, the female deposits a single egg.

The stem mother (fundatrix), after issuing from the egg, makes its way to the young leaves of the poplar, where it settles down on a petiole. Here a gall begins to form about it.

DESCRIPTIONS OF STAGES OF PEMPHIGUS POPULI-TRANSVERSUS AND ITS GALL

By C. P. GILLETTE

Examples of this species in the collection of the Colorado Experiment Station, which were collected by Mr. J. T. Monell at St. Louis, Mo., on October 2, 1907, agree in every particular with Riley's description of the winged form, except that the fifth joint of the antenna is not quite as cylindrical as the description would indicate. Specimens sent by Mr. T. H. Jones, of the Bureau of Entomology, which were collected from similar galls at Baton Rouge, agree perfectly with the Missouri specimens, and with specimens taken at many different times in Colorado. Winged and wingless "lice" taken by Mr. E. S. Tucker, then of the Bureau of Entomology, from the roots of turnips at Baton Rouge, on March 6, 1915; by Mr. F. B. Paddock, State Entomologist, on turnips, at College Station, Tex., on February 13, 1914; and by Mr. T. H. Jones on Brussels sprouts at Baton Rouge, on March 4, 1916, agree well in structural details.

The galls are widely distributed over Colorado upon the broad-leaved cottonwoods; but they are not abundant, except upon an occasional tree. It should be stated here that the sexupara of this species is separated with some difficulty from the same form of *Pemphigus betae*. In the latter species, however, the permanent sensorium of joint 5 is of the normal form and never broad and irregular, inclosing chitinous islands, as in *populi-transversus*, and the spur is distinctly longer.

FUNDATRIX, FIRST INSTAR.—Described from specimens reared by Mr. T. H. Jones at Baton Rouge, La., and taken from the galls on March 3, 1916.

Ground color pale yellow tinged with green; head black; wax plates dusky; length, 0.60 mm.; width, 0.23 mm.; antenna, 0.45 mm., 4-jointed, joints 1, 2, and 3 subequal in length, joint 4 with spur, almost as long as 2 and 3 combined and very stout, and with several transverse rows of small chitinous points; all femora stout, the greatest width of the hind femur nearly equalling one-half its length; six longitudinal rows of wax plates upon the dorsum of segments 1 to 6 of the abdomen, and four rows of larger plates upon the dorsum of the thorax, each plate bearing at least one short, stout hair; legs and antennæ deep shining black with a few short stout gray hairs (Pl. 81, A).

ADULT FUNDATRIX.—Described from living specimens taken at Fort Collins, Colo., by Mr. L. C. Bragg, Assistant Entomologist of the Colorado Experiment Station, on September 22, 1916, from galls on leaves of *Populus deltoides*. The opening of the gall was a straight, transverse, or somewhat diagonal slit, passing from one-half to two-thirds of the way across the gall, but not a narrow and protruded mouthlike or liplike

opening. The galls at this date appeared to be fully grown. Besides the stem mother, there were, in each gall, a few winged "lice" quite dark in color, a good number of pupæ of varying sizes, the small ones being quite pale in color, and numerous small larvæ which were very light colored and heavily tufted with white waxy threads. The old gall mother seemed to be the sole parent of the gall colony, all of which normally acquire wings.

The stem mothers were a yellowish sordid green in color, very plump, covered with a fine white powder; head, the entire legs, including coxæ, and tips of the antennæ dusky to blackish; antenna 4-jointed and very short, not as long as hind tibia, in length approximately 0.40 mm.; length of body 2.50 to 3 mm.; joint 3 distinctly longer than joint 4 with spur, the proportion being about as 3 to 2; length of hind tibia 0.50 mm.; eyes very small. There are upon the dorsum six longitudinal rows of rather large wax plates beginning upon the mesothorax and extending to the seventh abdominal segment. Upon the prothorax and the eighth abdominal segment the number of plates is reduced to four (Pl. 81, C, H).

The writer also examined, in connection with this description, numerous specimens taken in Louisiana by Mr. T. H. Jones, in California by Messrs. E. Bethel and George P. Weldon, in Arizona by Mr. Bethel, and on the eastern and western slopes of the mountains in Colorado by Messrs. L. C. Bragg and C. P. Gillette.

It seems certain that the wingless stem mother that starts the gall of this species early in the season normally continues to feed and reproduce until the leaves mature in the fall, all of her young acquiring wings and going in search of the alternate food plants of the family Cruciferae.

FUNDATRIGENIA MIGRANT FROM THE GALLS.—In color and general appearance like the winged sexupara from turnips and Brussels sprouts. The specimens examined average about 0.25 mm. shorter in body length. The differences in the antennal segments are quite marked. Joint 3 has from two to six transverse sensoria, the usual number being three or four. Joint 4 is the shortest and weakest and rarely has a small sensorium. Joint 4 being somewhat smaller, and joint 5 slightly larger than in the sexupara, the contrast in size of these segments is very noticeable. The permanent sensoria on joints 5 and 6 are very large and irregular, and even may be cut into two by projecting chitinous margins. They always have upon their surfaces small chitinous pieces, one or two on joint 5 and two to four on joint 6, each bearing one or more short hairs. Upon joint 6 this large irregular sensorium may extend from the base of the spur to the middle of the segment and is nearly always very irregular in outline. The proportions of the segments are about as follows: 1, 21; 2, 30; 3, 66; 4, 31; 5, 39; 6 with spur, 68. There are many irregularities in the antennæ of this species, one of which is the frequent union of segments 3 and 4 into one (Pl. 81, J).

WINGLESS VIVIPAROUS FEMALE.—Described from specimens taken by Mr. T. H. Jones at Baton Rouge, La., from the roots of Brussels sprouts, on April 2, 1917.

General color sordid pale yellow, with head, antennæ, and legs dusky brown to blackish; tarsi and eyes black; length 2.50 mm.; width 1.60 mm.; antenna 0.45 mm., joints 3, and 5 plus spur, subequal; joint 4 much the shortest, being less than one-half as long as joint 3; beak barely attaining second coxæ; hind femora and tibiae

each 0.55 mm.; body and legs very free from hairs; apparently no wax glands on the body (Pl. 81, E, I).

PUPA.—Almost uniform pale lemon yellow with slight greenish tinge on abdomen and a shade of flesh color upon the thorax; wing pads very slightly dusky along the outer margins; head, antennæ, and all the legs dusky; eyes black.

WINGED SEXUPARA.—Described from living specimens taken by Mr. T. H. Jones, Baton Rouge, La., on March 21, 1917, bred from Brussels sprouts, and from preserved specimens from Mr. F. B. Paddock, College Station, Tex., which were taken on February 2, 1913; from Mr. E. S. Tucker, Baton Rouge, La., taken on March 5, 1915, on turnips; and from one specimen taken by Mr. L. C. Bragg, near Fort Collins, Colo., on watercress (*Roripa* sp.) on August 31, 1917.

Head, antenna, entire thorax above, mesothorax below, and entire legs black; wings slightly smoky, with subcostal vein black or blackish and heavy along the inner or lower margin of the stigma; abdomen sordid light greenish yellow without markings; body everywhere with a slight covering of gray powder; dorsum of abdomen covered more or less with a cottony secretion; length of body 2 mm.; wing 2.70 mm.; antenna 0.60 mm.; hind tibia 0.75 mm.; joints of antenna in following proportions: 1, 22; 2, 30; 3, 65; 4, 32; 5, 32; 6 with spur, 56, sensoria transverse, joint 3 with four to eight, usually five or six; 4 with two or three; 5 and 6, normally, with permanent sensoria only; spur near base of joint 3 distinct. Permanent sensorium on joint 5 usually very large, often inclosing one or two chitinous pieces as in fundatrigene. Nervures of wing dusky, the costal and subcostal being heavy and black; stigma blackish, nearly parallel sided, and about three times as long as broad (Pl. 81, D, K).

OVIPAROUS FEMALE.—Described from a number of specimens deposited in a cage in the laboratory by specimens sent by Mr. T. H. Jones, from Baton Rouge, La.

General color buttercup yellow, with head, antennæ, and legs whitish and very transparent; a little dusky on the vertex; eyes black; antennæ short, 0.15 mm. long, 4-jointed, joints subequal, the first, and last including the spur, longest; length of body 0.70 to 0.90 mm. (Pl. 81, G).

EGG.—Oblong oval, glistening, varying in color from dull white to yellow. Ten eggs, deposited in the laboratory, averaged 0.54 mm. in length, ranging from 0.48 to 0.57 mm., and 0.21 mm. in width, ranging from 0.19 to 0.24 mm.

MALE.—Described from specimens born along with the oviparous females.

The males differ from the females by being pale yellowish green in color, more slender in form, and a little shorter, 0.60 to 0.65 mm. long, the legs very stout (Pl. 81, F).

GALLS.—Riley described the gall of this species as follows:

Formed upon the petiole near the base of the leaf of *Populus monilifera* and *P. balsamifera*. An elongate-oval swelling, causing the curving and broadening of the petiole, and opening on the opposite side by a transverse slit, with a whitish, slightly thickened, and elevated margin, recalling human lips.

The writer has studied large numbers of galls of this species from Colorado and Louisiana, the latter collected by Mr. T. H. Jones. When fully grown, they vary normally from about 12 to 18 mm., extra sizes attaining 20 or even 25 mm. in their greatest diameter, which is usually

in the direction of the petiole of the leaf. The fundatrix, or stem mother, soon after hatching from the egg, locates upon the petiole of a very young opening leaf, causing it to curve and thicken, and form a transverse groove (Pl. 81, B, a, b, c) at the point of attack. The petiole continues to thicken, and the groove to deepen, forming a pit or groove which carries the "louse" with it, and the two margins or lips gradually meet, inclosing the "louse" in a spacious cavity. The mouth or slit is usually transverse, but may be turned more or less in a vertical position, and the margins may, or may not, be thickened or protruded By the time the inmates become mature and ready to fly, the lips separate enough to allow the "lice" to pass out in search of the alternate food plants. The expanded petiole of the leaf can be easily seen extending along the convex surface of the gall opposite the mouthlike opening. (For typical forms of this gall, see Pl. 82; 84, A–E.)

The gall of this species is readily separated from the galls of *Pemphigus populicaulis*, which have a long curved opening formed by the twisting of the petiole upon itself, or from the galls of *P. populi-ramulorum*, which develop upon the side of tender growing twigs (Pl. 84, A–E).

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[1] More recently the writer has learned that as early as 1902 Dr. F. H. Chittenden recorded the receipt of specimens of an unknown species of Pemphigus from Texas. These were sent by Mr. S. A. McHenry, of the Beeville Substation of the Texas Experiment Stations, on February 14, 1901, with the information that the species was doing injury to the roots of cabbage in the vicinity of Beeville, some of the fields being reported as totally destroyed. (CHITTENDEN, F. H. SOME INSECTS INJURIOUS TO VEGETABLE CROPS. U. S. Dept. Agr., Div. Ent., Bul. 33, n. s, p. 79. 1902.)

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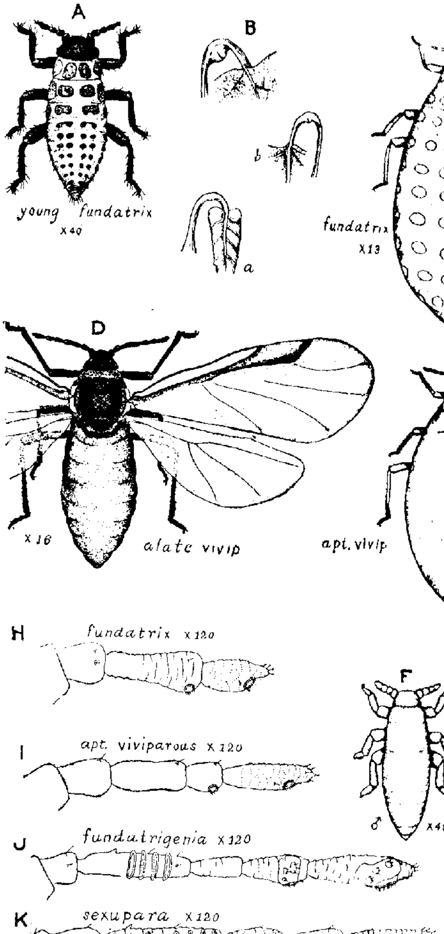
PLATE 81

Pemphigus populi-transversus:

A.—Young fundatrix, first instar. ×40.
B, a. b, c.—Beginning of galls on cottonwood leaves.
C.—Adult fundatrix with cottony secretion removed. ×13.
D.—Winged sexupara from roots of Brussels sprouts. ×16.
E.—Wingless virgogene from roots of Brussels sprouts. ×13.
F.—Male. ×40.
G.—Oviparous female. ×40.
H.—Antenna of fundatrix. ×120.
I.—Antenna of wingless viviparous female from Brussels sprouts. ×120.
J.—Antenna of winged fundatrigenia from gall. ×120.
K.—Antenna of winged sexupara from Brussels sprouts. ×120.
Drawn by Miss Miriam A. Palmer.


A
young fundatrix
x 40
B
b
a
C
fundatrix
x 13
D
x 16
alate vivip
E
apt. vivip
x 13
H
fundatrix x 120
I
apt. viviparous x 120
J
fundatrigenia x 120
K
sexupara x 120
F
♂
x 40
G
♀
x 40


A

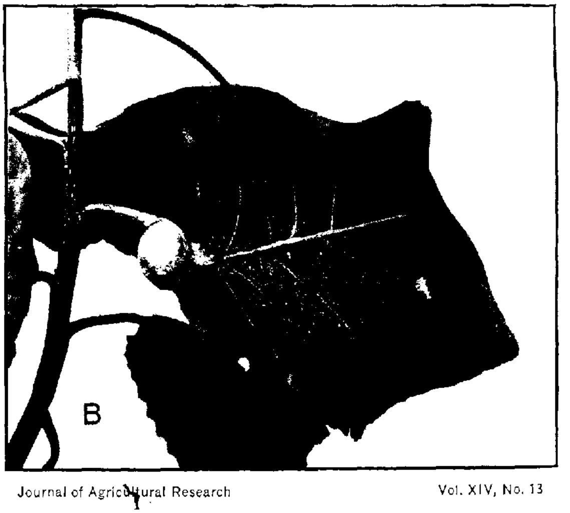

B

PLATE 82

Pemphigus populi-transversus:

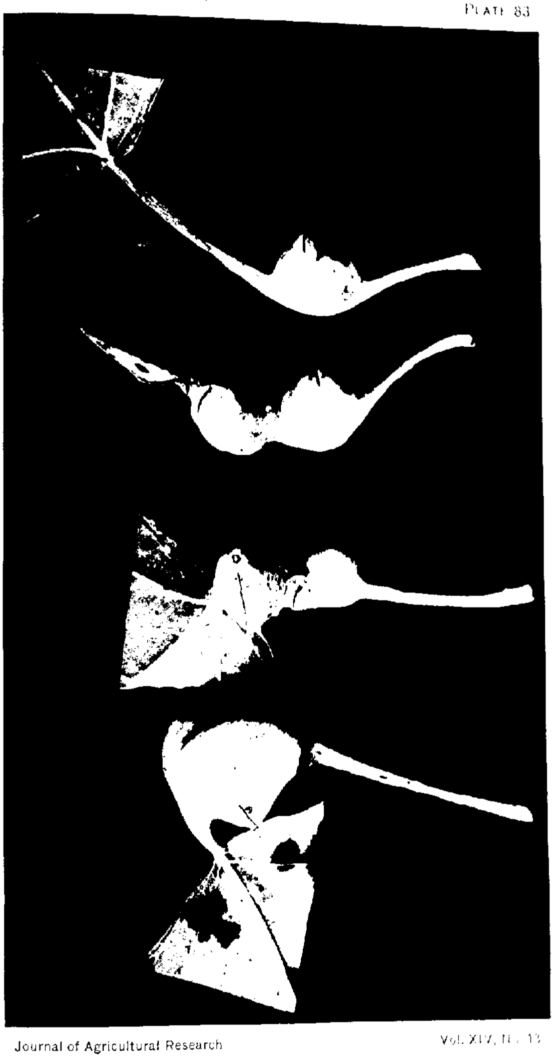
A.—Gall on poplar cutting. Cuttings were collected before the buds began to swell and were planted in sand in flowerpots kept in a greenhouse at Baton Rouge, La. Young stem mothers of *Pemphigus populi-transversus*, obtained indirectly from winged female migrants from roots of crucifers, were placed on growing cuttings on March 23, 1916. The photograph, taken on May 27, shows pot containing cuttings. One developing gall can be seen on twig at upper left.

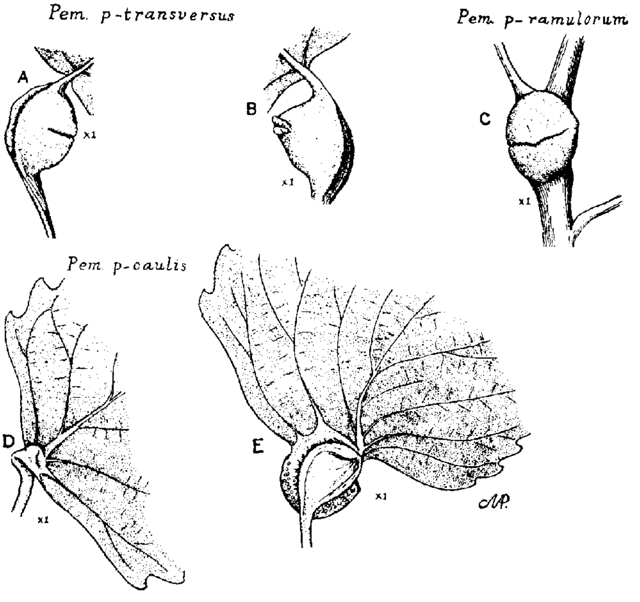
B.—Gall shown in A, enlarged to nearly natural size. Beside it can be seen the slit of a small gall which has failed to develop to any considerable extent.

PLATE 83

Pemphigus populi-transversus:

Variation in size of galls and location on leaf petioles of *Populus deltoides*, Baton Rouge, La., September 15, 1916. About natural size. Shows lips of galls all protruding, and in some cases thickened.




Pem. p-transversus
Pem. p-ramulorum
A
x1
B
x1
C
x1
Pem. p-caulis
D
x1
E
x1

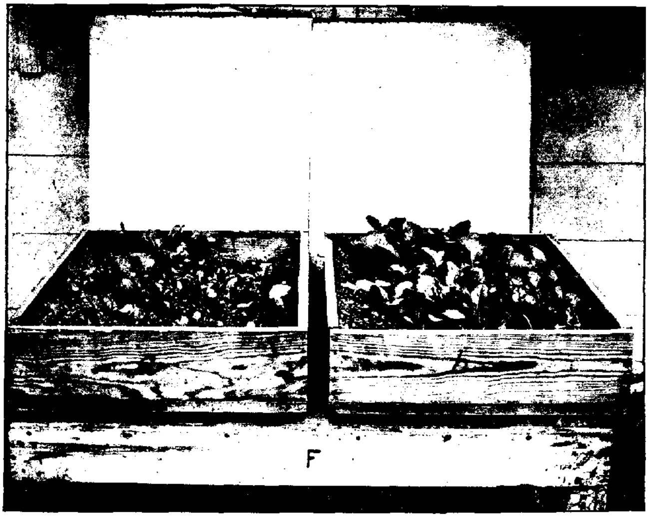

F

PLATE 84

A.—Gall of *Pemphigus populi-transversus*, lips not protruding.

B.—Gall of *Pemphigus populi-transversus*, lips protruding.

C.—Gall of *Pemphigus populi-ramulorum.*

D.—Beginning of gall of *Pemphigus populicaulis.*

E.—Full-grown gall of *Pemphigus populicaulis.*

All natural size. Drawn by Miriam A. Palmer.

F.—Turnip seedlings, showing injury by *Pemphigus populi-transversus: a*, infested; *b*, control. Turnips were planted at Baton Rouge, La., on September 16, 1916, in boxes having cloth-covered tops. Galls of *P. populi-transversus* were placed in box a on September 28. Photographed on October 13, at which time the wingless form of the aphid was abundant on roots of plants in box a. No root aphids found in box b. Note difference in growth of plants in the two boxes.

PLATE 85

Pemphigus populi-transversus:

F.—White cottony secretion at roots of Brussels sprouts due to presence of *Pemphigus populi-transversus*. Photographed at Baton Rouge, La., on February 10, 1916.

STEM LESIONS CAUSED BY EXCESSIVE HEAT

By Carl Hartley

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WHITESPOT INJURY

During work on the damping-off disease of pines (*Pinus* spp.) in 1909 the writer noticed in a nursery at Halsey, in the Nebraska sand hills, a type of disease closely corresponding to the old published descriptions of damping-off. The stems of very young seedlings developed shrunken areas at the soil surface. Commonly the entire stem was constricted by the lesion, the seedling fell over, and died. The writer has previously referred to this type of injury as "whitespot" (*4, p. 5*).[1] Close examination showed that this trouble differed in several ways from the type of damping-off which the writer and assistants have produced by inoculation with common pine-seedling parasites (*Pythium debaryanum* Hesse, *Corticium vagum* B. and C., and species of Fusarium). The primary whitespot lesions were in all cases limited to the stems, and usually just above the ground line. The whitespot lesion is very light in color, and this characteristic color continues to the very edge, making a sharp line of demarcation from the healthy tissue. Lesions may continue definitely limited for some days, and the upper stem and cotyledons remain turgid. In this early stage most cases of whitespot injury are easily distinguished from damping-off. Typical damping-off in porous soils is primarily a rootrot, which may attack above the ground line, but which more commonly attacks below. Damping-off lesions caused by any of the above-mentioned fungi or by *Botrytis cinerea* vary in color at different stages, gradually shading into the tissue still unaffected, and progress continuously both upward and downward.

This whitespot injury was at first supposed to be merely a special type of damping-off. Cultures made from the whitespot lesions failed to develop regularly any recognizable parasites, while most of the parallel cultures from lesions of the rootrot type yielded *Pythium debaryanum*. *Alternaria* sp. was the only fungus commonly obtained from the white spots.

Further examination showed that, in cases where whitespot lesions affected one side of the stem only, it was nearly always the south or southwest side. On seedlings which had been girdled, the lesions, if at all asymmetrical, extended higher on the south than on the north side. The south margins of the seed beds, imperfectly protected by the shade

[1] Reference is made by number (italic) to "Literature cited," pp. 603-604.

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frames, contained more whitespot lesions than other parts of the beds. In a single bed left entirely without shade, most of the seedlings died from whitespot. These observations indicated insolation as at least a contributory cause of whitespot injury. The nursery practice involved as little watering as possible during the damping-off period. In plots given somewhat more frequent watering than the general beds, subsequent counts showed only three-fifths as much whitespot injury as elsewhere. During the three succeeding years, the nurserymen gave the seed beds much more frequent watering and more careful shading than in 1909. In careful examinations during these three years only occasional cases of whitespot were found.

The foregoing data all pointed to a physical rather than a parasitic cause for whitespot injury. Heat and light were the physical factors toward which suspicion was directed. The temperature in the surface layer of soil in the seed beds, even under the half-shade of the lath frame, was found to go as high as 52° C. The apparent preventive effect of frequent watering was believed to be due to the lowering of the soil temperature.

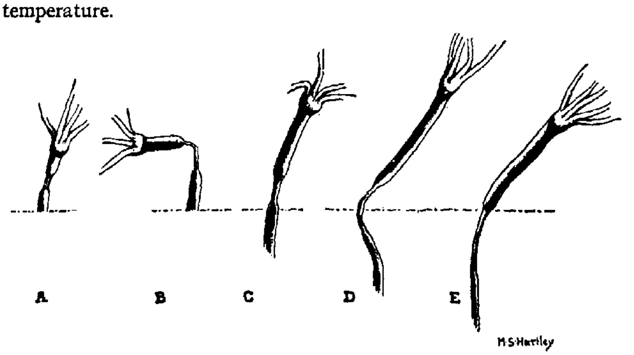

FIG. 1.—Lesions on seedlings of *Pinus ponderosa:* Seedlings A and D were injured by the sun's rays condensed by a lens. B was injured by a hot wire, C by an incandescent lamp, and E by the direct sun. The leaves remained turgid for 5 to 11 days after the lesions were produced. The horizontal line indicates the location of the soil surface at the time the seedlings were subjected to heat. Natural size.

In tests conducted at Washington, D. C., with seedlings of *Pinus ponderosa* typical whitespot lesions have been artificially produced. Five representative experiments are described in the following paragraphs and are illustrated in figure 1.

SEEDLING A.—The stem was subjected for less than two minutes to sunlight passed through a condensing lens at the point indicated by the constriction. The stem immediately collapsed and bent over at the point of the lesion. The soil around the root at the time was dry, and the seedling was distinctly wilted before it was heated. Later the pot was watered, and the seedling restored to a nearly vertical condition by propping. Turgor returned and was maintained for a week, but was followed by the decay of the root and wilting of the plant without extension of the original lesion (fig. 1, A).

SEEDLING B.—A heated wire held 1.5 mm. to the right of the stem for five minutes resulted in the lesion shown in figure 1, B. The plant remained turgid for 11 days and a whorl of new leaves appeared. The stem at the soil surface then decayed, apparently from a root infection, bearing a mat of pink spore masses.

SEEDLING C.—A Mazda incandescent lamp placed beside and slightly above the seedling for nearly two hours killed the tips of the cotyledons and produced a lesion just above the soil surface, without perceptible injury to intermediate tissue. In its location this lesion was thoroughly typical of those commonly found in the seed beds. Five days later fungus hyphæ appeared on the lesion, and the stem broke over at that point. The lesion then progressed both up the stem and into the root, and the seedling wilted. Spores of *Alternaria* spp. were promptly produced on placing the seedling in moist chamber (fig. 1, C).

SEEDLING D.—The stem and surrounding soil were subjected for three minutes to sunlight through a condensing lens, striking both the stem and soil surface at angles of about 45°. The resulting lesion extended about 3 mm. below the soil surface and 4 mm. above it. Mechanical support was required to keep the seedling from falling. Hyphæ soon appeared on the lesion at the soil surface, and in eight days after treatment wilting occurred, a species of Alternaria fruiting on the lesion in moist chamber (fig. 1, D).

SEEDLING E.—On a hot day, seedlings planted in loam in a 3-inch pot were placed immediately south of a brick wall. At night the seedling shown in figure 1, E, and another seedling were leaning slightly, but were apparently uninjured. An examination of the underground parts showed that these were distinctly shriveled from the soil surface to a point 10 mm. below; a third seedling in the pot, still erect and normal in appearance, was also constricted just below the soil surface. The plants were repotted and kept under observation. The tops of all remained entirely healthy for several days. At length the lesion began to extend up the stem, and on the eighth day wilting occurred. The entire root and 10 mm. of the stem above the soil line had become involved in the original lesion. Spores of species of Dactylosporium, Alternaria, and Fusarium appeared on the lesion in moist chamber.

Numerous seedlings of the same original lot were kept in the same room as the seedlings listed above during the period of the tests. None developed lesions which could have been mistaken for whitespot.

In all of the seedlings whose stems were heated directly the lesions were at first a dark grayish green, changing in 24 hours to the light color and shriveled appearance characteristic of whitespot lesions on seedlings in the nurseries. The immediate darkening is supposed to be due to the filling of the intercellular spaces with cell sap, while the ultimate light color presumably indicates the loss of liquid from both the intercellular spaces and the lumina of the cells and its replacement by air. In all cases the lesions remained definitely limited for several days, and were then extended, apparently as a result of infection by fungi not commonly capable of attacking uninjured plants. It appeared that in most cases neither the heat nor the fungi later entering the lesions stopped conduction or evolved toxins in sufficient quantity to cause the death of the leaves, as reported for another plant by Overton (*11*). Wilting finally occurred, it is believed, only when fungi entering at the lesion or at some point below it had penetrated the absorbing portion of the root. It may be remarked that in many cases of damping-off caused by the

ordinary seedling parasites, wilting probably occurs only after the absorbing portions of the root are invaded by the parasite.

The limitation of the whitespot lesions to the stem just above the soil surface in seedling C and in most of those observed in the nurseries, indicates that the combined radiation from the heated soil and from the sun direct ordinarily results in a temperature in the stem at that point higher than the temperature in the surface soil. In an experiment not described above, in which the source of heat was directly above the seedling, the lesion was, as might be expected, just below the soil surface rather than above it. The pot containing seedling E was also so placed that the sun's rays were more nearly perpendicular to the soil surface than in level seed beds, with the same result. It is probable that in at least some cases heat lesions will occur in the nurseries partly or entirely below the soil surface, as in seedlings D and E. It will be impossible, by any ordinary method of field observation, to distinguish from damping-off cases such as that of seedling E. Both the angle of the sun's rays, and the absorbing, conducting, and radiating capacity of the soil and of the stem will, of course, help determine whether the stem will be hottest above or just below the soil surface.

Münch (*8*, *9*) has described the same type of injury to tree seedlings in Germany, attributing it positively to heat at the soil surface. With a thermometer having a thin, flat bulb he obtained very high temperatures in the surface soil (*10*). Others have also reported surface temperatures in unprotected soil from 55° to 68° C., or even higher (*2, p. 55; 7, p. 13; 12; 17*). Münch made an incubator test in which coniferous seedlings survived for two to three hours at temperatures not exceeding 52°, but were killed by maxima of 54° to 55° C. This seems in general to agree with the temperatures reported as being fatal to most growing plants.

Typical whitespot injury to seedlings has been found in several different States, though nowhere has it been observed to cause as heavy losses as at the Nebraska nursery, where it was first seen. Whitespot is not limited to conifers. In the vicinity of the Nebraska nursery an examination of fields of rye (*Secale cereale*) and cowpeas (*Vigna sinensis*) showed that both were affected in the seedling stage in much the same way as the pines. In the cowpeas the localization of the white, constricted lesions just above the soil surface was very marked. Plate cultures yielded no fungus suspected of parasitism. The rye seedlings were affected in the same way, though constriction was less in evidence than in the more fleshy plants. The relation between the disease and exposure to sun was very evident in the case of the rye. In the level portion of the field a moderate proportion, perhaps 5 per cent, of the shoots were affected. Where a dead furrow crossed the field from east to west this uniform distribution of disease was broken. On the wall of the dead furrow having a north exposure the disease was not noticeable.

On the other wall, exposed to the south, the percentage of affected shoots was much greater than on the level surface of the rest of the field. Wind action was not excluded as a possible cause of the cowpea lesions, but the protected location of the nursery, so far as south winds are concerned, made the evidence rather conclusive that insolation rather than wind was responsible for the lesions on the rye. Münch (*8*) reports whitespot on maple, vetch, and peas, and believes that in some cases germinating seeds, as well as seedlings which have already broken soil, are killed by overheated soil.

Whitespot is not always fatal, even when the lesion girdles the stem. Two seedlings of *Pinus ponderosa* which had been girdled by definite whitespot lesions, slightly shrunken but not severe enough to cause breaking over, were marked for later observation. At the end of the season the lesions had disappeared and the plants seemed in every way normal. In leaf lesions due to heat, Sorauer (*15, p. 638*) has after several weeks observed a regeneration of chloroplasts in slightly affected tissues.

All things considered, whitespot lesions are believed to be caused mainly by excessive heat. While light as such may possibly take part in some cases, it evidently does not enter into all cases of injury. The relative unimportance of light as distinguished from heat is indicated by the numerous lesions under slat frames, the extension of all serious lesions to the north sides of stems, and the experimental production of lesions below the soil surface (seedlings D, E, and others). The preliminary experiments here reported were mostly at excessive temperatures, and absolute proof that heat alone is the cause of the common lesions in the seed beds must await further experiments at temperatures which more commonly occur in nature.

BASAL LESIONS ON SEEDLINGS SEVERAL MONTHS OLD

A type of trouble which is probably related to the whitespot described in the foregoing was observed in 1915 in the seed beds of a nursery of the United States Forest Service, located at an elevation of 7,300 feet in the Wasatch Mountains, Utah. The plants affected were spruce and Douglas fir which had been raised from seed the preceding year. They had made a normal height growth during their first season and remained green throughout the winter under a heavy coating of snow which covered them for more than five months. Two or three weeks after the snow melted many of the seedlings began to turn yellow and ultimately died. Examination showed dead bark, beginning at the soil surface and extending up the stem from 3 to 9 mm. In many cases the lesion extended farther up the stem on the south than on the north side, and on some seedlings lesions were found which were entirely limited to the south side of the stem, and had started to heal over from the edges. In no case was there found any such swelling above the lesion as occurs above stem-girdle lesions on older stock. In many of the advanced cases the cortex

from the base of the stem was partly or entirely gone. Careful examination, however, indicated that even these lesions could not be attributed to any biting insect. The affected seedlings were distributed rather evenly over the beds, but in no case were any diseased plants found immediately north of posts, or on the north exposed slopes at the ends of beds, where the seedlings were somewhat protected from the sun during the hottest part of the day. Spruce, a more shade-loving tree than Douglas fir, also suffered more from the disease. The observations made indicate that the death of these seedlings was due to whitespot lesions occurring during the latter part of the preceding summer. The altitude of the nursery at first thought renders it improbable that excessive heat should have been concerned in causing the injury. While, of course, the temperature of the air at such elevations is never very high, the heat of rocks and gravel exposed to the sun at high altitudes is well known. Tubeuf (*17*) reports a surface soil temperature of 60° C. (140° F.) at an elevation of 10,000 feet in Yellowstone Park, 200 miles directly north of the Wasatch region.

BASAL STEM-GIRDLE ON OLDER STOCK

Münch and others (*5, 7, p. 13; 12, 13, p. 397; 14, 15, p. 638*) have further attributed to excessive surface temperatures of the soil the "*Einschnürungskrankheit,*" or stem-girdle, of older nursery stock or young forest trees of both conifers and broad-leaved trees. Dr. B. T. Galloway, of the Bureau of Plant Industry, told the writer that he had found basal lesions on young willows at Chico, Cal., which he attributed to excessive heat. In conifers this disease involves death of the base of the stem of 2-to-4-year-old seedlings and transplants. Lesions are definitely limited, and the swollen growth of the stem just above the lesion, which results from the girdling and interference with food movement, gives an appearance of constriction at the lesion itself. This disease is figured by Tubeuf (*16, p. 492*) and ascribed to *Pestalozzia hartigii*. Hartig (*3*) had originally ascribed it to the freezing of thin pools of water standing on the surface of the beds. The parasitism of *P. hartigii* has failed of confirmation (*1*), and Tubeuf (*17*) now seems to favor the view of Münch, that heat of the soil is responsible. This disease has been found at widely separated points in the United States. The writer has seen what appeared to be stem-girdle on two species of the white and three of the pitch pines, two spruces, *Abies concolor* (Gord) Parry, *Pseudotsuga taxifolia* (Poir.) Brit. *Thuja* sp., and *Juniperus* sp.[1] Its appearance and its ability to attack representatives of so many different genera favor a nonparasitic diagnosis. The thicker cortical tissues of the woody stems should make the cambium slower to reach maximum temperature, and prevent quite as high a temperature being reached, as in the case of the younger stems,

[1] Several of these observations as to coniferous species affected were communicated by Dr. J. V. Hoffman, of the Forest Service, and confirmed by the writer's examination of his specimens.

which are subject to whitespot. It is worthy of note that no cases of stem girdle have been found in four seasons' examinations of the Nebraska nursery at which whitespot has been so frequent. The heat hypothesis nevertheless seems the best explanation of stem-girdle so far offered. Tubeuf's experiments with warm water (*18*) are of interest in this connection as indicating that moderately high, long-continued temperatures do not necessarily kill simply by their drying effect, as has sometimes been claimed.

LESIONS ON UPPER PARTS OF STEMS

Older 2-needled pines and herbaceous plans as well have been observed by the writer to develop typical shrunken, definitely limited whitespot lesions on young growth of the upper parts of their stems, usually at points where an abnormal bend had made the surface nearly perpendicular to the sun's rays. Such lesions seldom girdle stems, and are rarely, if ever, of economic importance.

In the case of *Pinus strobus* the unusual amount of attention which pathologists have given it in the last two years has resulted in the finding by blister-rust scouts at five different places of yellowish lesions on young stems, sunken, and in all or nearly all cases limited to one side of the stem. Sections made by Dr. R. H. Colley, of the Bureau of Plant Industry, showed a collapsed condition of the tissues, but with absence of mycelium. Observations on these lesions in northern Wisconsin by Mr. R. G. Pierce, of the Bureau of Plant Industry, showed that practically all occur on the upper sides of bent shoots or on the west sides of vertical shoots, though a single case was found on the north side. The greater number of the affected plants were on the west sides of the nursery beds.

Most of these lesions are presumed to be due to heat. In the few cases in which soft young shoots of *Pinus banksiana* have been found girdled and bent over at the point of the lesion, mechanical bending is also suggested as a possible cause.

LESIONS DUE TO EXCESSIVE BENDING

Following cold weather with high winds, pine seedlings 1 to 2 weeks old have been found with white constricted basal lesions in some ways not like those produced by heat. There is reason to believe that these are due to the constant bending in high wind, at length causing the collapse of the cortical tissues without the stress being sufficient at any one time to rupture the epidermis or break the fibrovascular bundles. This is apparently an analogous case to the death of tissue between the veins of sugar maple leaves exposed to storm (*6, p. 27-28*). A few cases of lesions on shoots of older pines have already been mentioned as possibly the results of mechanical bending rather than of heat, and it is entirely possible that the whitespot lesions observed on cowpea seedlings should

be attributed to bending rather than to heat. Basal stem-girdle lesions found on 4-months-old wild olive seedlings (*Elaeagnus* sp.) and characterized by very slight vertical extension may be due to excessive bending. However, stem lesions caused by bending without breakage are not believed to be common enough to give rise to serious confusion with those caused by heat.

PREVENTIVE MEASURES

Assuming the correctness of the hypothesis that most of them are caused by heat, the logical procedure for preventing whitespot and the basal lesions on older stems is to avoid soils especially liable to overheating, and in established nurseries where trouble occurs, to artificially prevent heating. Soil with loose texture or dark surface is presumed most likely to overheat at the surface. Shading and frequent light watering have already been found helpful in preventing whitespot. Encouraging free air movement and artificially compacting the soil to increase its conducting capacity have been suggested as having prophylactic value.

SUMMARY

(1) Very young seedlings of conifers and certain other plants were found dying in large numbers in a Nebraska nursery from a disease which, because of its characteristic lesions, the writer has called "whitespot." The trouble has been found, though less commonly, in other localities. It is distinct from the common damping-off disease, but resembles it so closely that it is very likely to be confused with it. The lesions do not seriously interfere with the upward movement of water.

(2) The location of the whitespot lesions on the stems, their observed relation to insolation and to dry surface soil, and the production of typical lesions by artificial heating, indicate excessive heat as the cause of most of the whitespot trouble.

(3) The observation in the surface soil in the seed beds in question, and by other investigators in other places, of temperatures well over 50° C., with reported maxima as high as 68°, further substantiates the hypothesis that whitespot is due to excessive heat.

(4) Killing lesions on stems of older conifers ranging in age from several months to 4 years, are also attributed to heat. The causal importance of heat in these lesions on woody or semiwoody stems is less well established than in the case of whitespot. Further experimental work at temperatures such as actually occur in nature is necessary to settle finally the pathological importance of high soil temperature.

(5) Lesions involving young cortex and resembling those attributed to heat are probably in some cases caused by repeated bending in heavy wind without visible breakage. These are believed to be too rare to give rise to serious confusion.

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WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1917

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The interesting history of the many successful introductions of beneficial insects into Hawaii includes little exact data on the activities of these insects during the first few years after liberation. It is during this period that some immediate adaptations may be necessary to enable the insects to conform to a new environment, and many unexpected fluctuations may occur between the various species introduced to attack the same host before a balance is reached among them that can be expected to remain fairly constant during the years to follow. This period, then, is of much biologic interest. Apart from this, insufficient data have been published which convey accurate information concerning the work of these insects and the enormous check constantly being exerted by them over destructive pests, without which many forms of agriculture could not be conducted with profit. Aside from the aid which the entomologist can give the farmer in distributing beneficial insects over the earth beyond their natural barriers, it is his duty to obtain informing data on the kind and extent of assistance that is continually being rendered to agriculture through the work of beneficial insects, both native and introduced.

The spread and value of the parasites introduced into Hawaii to attack the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) have been watched and recorded yearly since the first liberations in 1913.[1] With the object of continuing this unbroken series of data and of again informing those interested of the degree of success attending these introductions, the following data are given to indicate the work of the established parasites throughout the year 1917, and the extent of fruit-fly injury caused to fruits in the Territory during that year.

During the year there was a rather heavy infestation of several varieties of fruits, some kinds being badly infested, as shown in Table I. From 913 peaches (*Amygdalus persica*) collected about Honolulu a total of 13,904 larvæ developed, or an average of 15.2 maggots to each fruit. Hardly a peach was sufficiently free from maggots to be edible. From

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PEMBERTON, C. E., and WILLARD, H. F. FRUIT-FLY PARASITISM IN HAWAII DURING 1916. *In* Jour. Agr. Research, v. 12, no. 2, p. 103-108. 1918.

17,960 kamani nuts (*Terminalia catappa*) collected during the year, 143,246 maggots were obtained, or an average of 8 to each fruit. Fortunately most other fruits were somewhat less infested than these. Though the infestation is considerable, the parasitism, as shown in Tables II and III, is very high in some cases, and during the year averaged 47.5 per cent for all fruits taken together, as determined from records on 72,139 larvæ.

TABLE I.—*Extent of infestation of host fruits by larvæ of Ceratitis capitata in Hawaii during 1917*

Host fruit.	Number of fruits collected.	Number of *C. capitata* larvæ emerging.	Average number of larvæ per fruit in 1917.	Average number of larvæ per fruit in 1916.
Indian almond (*Terminalia catappa*)	17,960	143,246	8.0	9.5
Mango (*Mangifera indica*)	648	5,250	8.1	1.7
Coffee (*Coffea arabica*)	58,272	45,788	.8	.5
Strawberry guava (*Psidium cattleianum*)	9,872	19,795	2.0	1.6
Black myrobalan (*Terminalia chebula*)	1,767	10,411	5.9	7.0
Peach (*Amygdalus persica*)	913	13,904	15.2	20.5
Rose-apple (*Eugenia jambos*)	1,115	9,811	8.8	5.5
Satin-leaf (*Chrysophyllum olivaeforme*)	6,905	23,745	3.4	2.0
French cherry (*Eugenia uniflora*)	21,554	21,911	1.0	.8
West Indian medlar (*Mimusops elengi*)	4,133	7,287	1.8	5.3
Yellow-wood (*Ochrosia elliptica*)	56	1,312	23.5	3.1
Kamani (*Calophyllum inophyllum*)	346	838	2.4	3.3
Yellow oleander (*Thevetia neriifolia*)	2,960	16,667	5.7	3.6
Carambola (*Averrhoa carambola*)	316	185	.6	1.3
Chinese orange (*Citrus japonica*)	8,050	14,090	1.8	3.1
Guava (*Psidium guajava*)	1,342	6,009	4.5	6.8
Loquat (*Eriobotrya japonica*)	1,980	5,204	2.6	....
Kona orange (*Citrus sinensis*)	350	1,600	4.6	....
Mandarin orange (*Citrus nobilis deliciosa*)	341	732	2.2	....
Sapodilla (*Achras sapota*)	104	488	4.7	....
White sapote (*Casimiroa edulis*)	244	1,845	7.6	....
Wampi (*Clausena wampi*)	117	24	.2	....

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii in 1917* [a]

Host fruit.	Month of collection.	Number of larvæ emerging during first 2–6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
	1917.						
Indian almond	January	677	7.5	44.3	....	1.3	53.6
Do.	February	690	6.1	16.8	....	1.3	24.2
Do.	March	302	10.3	22.2	....	7.3	39.8
Do.	April	342	10.2	22.8	....	3.5	36.5
Do.	May	1,035	11.4	34.3	0.3	.7	46.7
Do.	August	4,620	4.4	15.8	.2	3.5	23.9
Do.	September	5,148	5.0	46.8	.02	3.3	55.12

[a] Most of the fruits represented in this table were collected about Honolulu at low elevations. The coffee, however, was collected on the island of Hawaii, in addition to localities in Honolulu, and much of it came from points from 1,000 to 2,000 feet above sea level. The March collection of coffee came entirely from Kona on the island of Hawaii.

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii in 1917*—Con.

Host fruit.	Month of collection.	Number of larvæ emerging during first 2–6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
	1917.						
Indian almond	October	6,149	12.1	29.6	.3	15.2	57.2
Do	November	3,791	12.1	31.5	.5	30.3	74.4
Do	December	961	11.0	32.3	2.5	19.1	64.9
Mango	February	......	......	......	......	......	......
Do	June	567	2.8	14.6	.7	8.0	26.1
Do	July	207	3.9	15.0	2.9	12.6	34.4
Do	August	35	......	......	8.6	2.9	11.5
Coffee[a]	January	3,638	59.3	10.6	.8	......	70.7
Do	February	501	72.4	6.6	4.4	......	83.4
Do	March	1,722	50.0	33.2	5.9	......	89.1
Do	June	554	55.4	1.4	......	......	56.8
Do	August	22	......	9.1	45.5	......	54.6
Do	September	3,527	5.9	11.9	33.2	.03	51.03
Do	October	2,196	3.4	1.0	66.1	.05	70.55
Do	November	1,541	35.0	5.9	38.4	.5	79.8
Do	December	176	21.0	3.4	24.4	.6	49.4
Strawberry guava	January	18	16.7	33.3	......	......	50.0
Do	March	804	20.0	30.0	7.6	.1	57.7
Do	April	1,172	13.7	39.8	3.2	1.1	57.8
Do	May	603	12.3	50.7	1.7	1.8	66.5
Do	June	1,034	10.9	31.9	1.1	4.4	48.2
Do	July	939	6.1	74.4	......	.9	81.1
Do	August	220	11.8	15.5	23.6	12.3	63.2
Do	November	160	......	21.3	6.9	30.6	58.8
Do	December	267	......	57.7	3.4	4.1	65.2
Black myrobalan	January	355	8.2	5.6	4.2	34.6	52.6
Do	October	2,860	.9	.7	.5	3.9	6.0
Peach	April	263	.8	47.9	......	2.3	51.0
Do	May	833	3.6	37.8	......	9.2	51.5
Do	June	2,343	8.1	17.0	......	14.5	39.6
Do	July	1,391	6.3	18.0	......	44.8	69.1
Rose-apple	May	1,021	8.0	13.6	......	.1	21.7
Do	June	386	1.6	20.7	......	1.3	23.6
Do	July	274	.7	27.7	......	1.8	30.2
Do	August	358	.6	29.1	......	1.1	30.8
Satin-leaf	February	2,484	18.4	3.4	1.3	.9	24.0
Do	March	831	37.5	5.4	10.7	1.1	54.7
French cherry	January	1,258	13.4	19.5	5.9	.8	39.6
Do	February	290	13.8	31.4	3.8	1.4	50.4
Do	March	1,320	13.7	23.8	12.3	.5	50.3
Do	April	351	5.1	24.8	33.0	......	62.9
Do	May	346	6.7	9.5	39.0	.6	55.8
Do	November	143	12.6	6.3	40.6	......	59.5
Do	December	1,421	1.1	2.0	4.4	.1	7.6
West Indian medlar	June	475	5.3	6.7	.2	3.8	16.0
Do	July	174	.6	1.2	......	1.7	3.5
Do	August	478	.2	......	.2	......	.4
Do	September	78	1.3	......	......	......	1.3
Yellow-wood	June	153	......	5.2	......	......	5.2
Kamani	February	250	......	19.6	......	......	19.6
Do	March	94	......	57.4	......	......	57.4
Yellow oleander	January	7	......	......	14.3	57.1	71.4
Do	February	56	1.8	1.8	8.9	28.6	41.1
Do	March	17	5.9	5.9	......	41.2	53.0

[a] The June collection of coffee came from the Waianæ Mountains. *Opius humilis* was first established here, but recently *Diachasma tryoni* was liberated.

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii in 1917*—Con.

Host fruit.	Month of collection.	Number of larvæ emerging during first 2–6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
	1917.						
Yellow oleander	July	199	......	13.6	13.6	47.7	74.9
Do	August	1,679	.4	7.5	14.4	32.0	54.3
Do	September	294	1.7	2.0	8.5	25.8	38.0
Do	October	104	......	......	25.0	14.4	39.4
Carambola	December	59	8.5	5.1	......	6.8	20.4
Chinese orange	February	68	4.4	5.9	......	1.5	11.8
Do	March	188	6.4	.5	1.6	1.1	9.6
Do	April	210	6.4	14.6	.5	.9	22.4
Do	May	100	17.0	11.0	......	......	28.0
Do	June	278	13.3	8.3	......	2.9	24.5
Do	December	167	1.2	9.6	3.0	4.2	18.0
Ponay	June	129	.8	1.6	......	......	2.4
Do	July	386	......	1.3	.2	2.2	3.7
Do	August	852	.1	26.3	......	8.0	34.4
Guava	January	120	1.7	.8	......	......	2.5
Do	February	89	3.4	......	......	......	3.4
Do	March	100	1.0	......	......	3.0	4.0
Do	April	472	2.1	.2	.4	.2	2.9
Do	May	91	8.8	......	......	......	8.8
Do	July	83	6.0	3.6	......	48.2	57.8
Do	August	446	1.8	46.9	.2	1.3	50.2
Do	November	153	.7	......	......	......	.7
Loquat	January	19	10.5	21.1	31.6	......	63.2
Do	February	109	1.8	11.9	62.4	5.5	81.6
Do	March	441	7.3	6.8	73.5	5.7	93.3
Do	December	41	4.9	4.9	51.2	22.0	83.0
Kona orange	January	......	......	......	......	......	......
Do	February	14	......	7.1	......	......	7.1
Do	March	39	5.1	......	2.6	......	7.7
Do	May	14	7.1	14.2	7.1	7.1	35.5
Do	November	64	......	......	10.9	51.6	62.5
Mandarin orange	November	67	3.0	13.4	......	......	16.4
Do	December	50	......	12.0	......	4.0	16.0
Sapodilla	February	10	30.0	......	......	......	30.0
Do	March	43	16.3	......	......	......	16.3
Do	April	42	42.9	......	......	......	42.9
White sapote	May	396	9.1	1.0	......	1.0	11.1

Thus, as seen in Table III, nearly one-half of all the Mediterranean fruit-fly larvæ developing during the year were destroyed and this is entirely the result of parasitic importations. This achievement, solely due to the efforts of the Territorial Board of Agriculture and Forestry, is worthy of unusual commendation. Insufficient emphasis perhaps has been placed during recent years upon the utility of these parasites. The constant extinction of at least 45 per cent of all worms developing in fruit, apart from the destruction of this pest through other agencies already present in the islands, notable among them being the ant *Pheidole megacephala* Fabricius, without question greatly decreases the infestation of such fruits as the orange (*Citrus sinensis*) and certain

varieties of mango and avocado not ordinarily susceptible to prohibitive infestation except under conditions permitting an unchecked multiplication of the fly. This point has come to light during 1917, and the statement seems justified that a 50 per cent reduction in the numbers of the fly brings little relief to its favored host fruits, but that those fruits classed as unfavored hosts show a marked improvement in the degree of infestation, and some may become almost wholly free from larvæ. The propagation of such fruits and the encouragement of the parasitic method of control would seem to be the most favorable method of contending with this pest in Hawaii.

TABLE III.—*Total parasitism, by month, of all larvæ of Ceratitis capitata collected in Hawaii during 1917*

Month.	Number of larvæ.	Percentage of parasitism.					
		Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total in 1917.	Total in 1916.
January	6, 183	39. 0	15. 6	2. 0	2. 4	59. 0	6. 98
February	4, 568	20. 0	8. 6	3. 0	1. 3	32. 9	19. 5
March	5, 901	27. 1	22. 5	12. 6	1. 3	63. 5	14. 7
April	2, 861	9. 0	27. 6	5. 5	1. 2	43. 3	37. 64
May	4, 439	8. 8	26. 4	3. 4	2. 3	40. 9	26. 69
June	5, 919	11. 7	16. 3	. 3	7. 8	36. 1	27. 81
July	4, 125	3. 9	26. 6	. 9	19. 6	51. 0	18. 52
August	8, 726	2. 8	16. 4	4. 7	9. 2	33. 1	37. 5
September	9, 047	5. 2	31. 3	13. 2	2. 7	52. 4	45. 2
October	11, 309	7. 2	16. 4	13. 2	8. 4	45. 2	44. 3
November	5, 919	17. 2	22. 6	11. 6	20. 9	72. 3	44. 3
December	3, 142	5. 3	16. 7	5. 2	7. 0	34. 2	44. 1
Average, 1917	72, 139	12. 7	20. 3	7. 3	7. 2	47. 5	.....
Average, 1916	83, 304	17. 2	13. 3	2. 1	. 6	.....	33. 2

This 47 per cent reduction in the abundance of the fruit fly in Hawaii serves another purpose well worthy of mention. It is an important help contributing toward reducing the chances of its introduction to the mainland.

As shown elsewhere by the writers, the parasitism by the braconid *Opius humilis* Silvestri has been found highest during the coolest months of the year. Again during 1917 this was observed. The parasitism by *O. humilis* exceeded that of all of the other parasites combined in the months of January and February and was greater than that of any of the others taken separately during March. During the remaining months the parasite *Diachasma tryoni* was more abundant than the *O. humilis*. It is in the cool months of January, February, and March that the other parasites are so retarded that the *O. humilis* is enabled to gain a considerable foothold.

The average parasitism in all fruits by *Opius humilis* in 1915 was well above that of all the other parasites combined; in 1916 the total parasitism by it was 17.2 per cent, and by the others combined was 16 per cent; while during 1917 the other parasites had so reduced the *O. humilis* that the total by it was only 12.7 per cent and by the others combined was 34.8 per cent. This reduction of the Opius is proceeding slowly, but the species is not expected to be entirely annihilated. The coffee collections in the Waianæ Mountains at the head of the Waianæ Valley during 1917 were interesting. This was the only point at which *O. humilis* had been established when the collections were made. In February it was found parasitizing 89.4 per cent of the larvæ in the coffee; and in June 77.5 per cent were parasitized by it.

The parasite *Tetrastichus giffardianus* Silvestri was more abundantly recovered from fruit collections in 1917 than in any preceding year since its liberation in the islands in 1914. It was recovered, however, only in material collected about Honolulu. Its ability to penetrate to the interior of soft fruits broken or containing holes enables it when numerous to parasitize large numbers of larvæ in such fruits as the mango, the orange, or the common guava. The total average parasitism in larvæ from all fruits during the year was materially increased through the work of *T. giffardianus* in such fruits.

The total parasitism by all species during 1917 was 14.3 per cent higher than in 1916. The average infestation of all fruits combined was, however, not strikingly different from that of 1916.

As shown in Table III, the parasitism by *Opius humilis* is 4.5 per cent less than in 1916, while that of *Diachasma tryoni, D. fullawayi* Silvestri, and *Tetrastichus giffardianus* was 7.0 per cent, 5.2 per cent, and 6.6 per cent greater, respectively.

CORN-ROOTROT AND WHEATSCAB

[PRELIMINARY PAPER]

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In the progress of the investigations of rots of the root, stalk, and ear of Indian corn (*Zea mays*) being conducted by one of the authors (Hoffer) certain things have developed which have such an important bearing on certain phases of the wheatscab problem which is being investigated by the other authors that it has seemed desirable to publish a preliminary statement.

Field observations have shown a conspicuously greater abundance of wheatscab in fields where the wheat (*Triticum aestivum*) was grown immediately following corn that was infected with the Fusarium-rot of the root and stalk. This was especially true in Shelby County, Indiana, where wheat, according to a common practice, was sown in standing corn.

A similar condition was also noted in Dane County, Wisconsin, this summer, where spring wheat was grown immediately following a corn crop. Both in Indiana and in Wisconsin under these conditions abundant development of perithecia of *Gibberella* spp. was found on the old cornstalks remaining in these fields. These perithecia were mature and well filled with viable ascospores at the time when the wheat, in all cases observed, was in head.

Water suspensions of these ascospores both from Indiana and from Wisconsin cornstalks gave positive results when used as inocula on wheat heads. The inocula were applied by means of an atomizer spray. In some cases the heads were subsequently covered with glassine bags for three days, and in other cases no coverings were used. In all cases positive infections were obtained, while the controls sprayed with sterile water and similarly treated remained unaffected. The appearance of the infected heads thus artificially inoculated was identical with that of wheat heads naturally infected with scab.

Cultures from *Gibberella* spp. on old cornstalks have also proved virulently parasitic on the roots of corn plants grown both in large, sterile agar tubes and in sterilized pots of soil.

Similar results on both wheat and corn have been obtained by using cultures from naturally infected wheat heads.

The organisms from both sources have also been found to be similar morphologically. In view of the facts developed by this evidence, it seems certain that these are intercrop parasites which are of great impor-

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tance in developing control measures for one of the rots of the root, stalk, and ear of corn and for scab of wheat. As both corn and wheat are such highly important food crops, it is imperative that the investigations bearing on any of these disease problems should be pushed forward with utmost vigor at the present time.

While the data are as yet somewhat fragmentary, it seems evident that, in order to lessen the losses from these diseases on corn and wheat, it is necessary to recognize this intercrop parasitism and develop field practices accordingly. In general, the use of the best-adapted, disease-free seed on clean soil should be practiced. The details of control measures for these diseases of corn and wheat are as yet not worked out, and no simple ones are evident. A crop rotation avoiding wheat following diseased corn is undoubtedly important, unless the cornstalks can be cut close to the ground, removed, and the remaining stubble plowed under before the wheat is planted. Badly scabbed wheat should not be used for seed. Ordinary seed treatments will not control wheatscab; hence, only clean seed on carefully prepared clean soil should be used.

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